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COMPARATIVE *IN VITRO* STUDIES OF FURAZIDIN AND NITROFURANTOIN ACTIVITIES AGAINST COMMON UROPATHOGENS INCLUDING MULTIDRUG-RESISTANT STRAINS OF *E. COLI* AND *S. AUREUS*

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Abstract: Urinary tract infections caused by wide range of pathogens including gram-negative and gram-positive bacteria as well as fungi are a severe public health problem. The predominant causative agent of both uncomplicated and complicated urinary tract infections is *Escherichia coli*. In an era of increasing bacterial resistance to antimicrobial agents and a high prevalence of multidrug-resistant (MDR) strains in community- and hospital-acquired infections, the re-evaluation of older generations of antimicrobial agents, such as nitrofuran derivatives, seems to be a reasonable approach. The aim of the study was to evaluate furazidin activity against common uropathogens in comparison to nitrofurantoin and other selected antimicrobial agents, routinely used in the treatment of urinary tract infections. Furazidin exhibited lower MICs than nitrofurantoin when tested against gram-negative and gram-positive bacteria including clinical MDR *E. coli* and methicillin-resistant *Staphylococcus aureus*. The MICs for furazidin ranged from 4 to 64 mg/L for Enterobacteriaceae strains, from 2 to 4 mg/L for gram-positive cocci, and 0.5 mg/L for anaerobic bacteria. The MICs for nitrofurantoin ranged from 16 to 64 mg/L for Enterobacteriaceae strains, from 8 to 64 mg/L for gram-positive cocci, and 4 mg/L for anaerobic bacteria. In addition, both nitrofurans displayed better activity against the tested bacterial strains than ciprofloxacin, fosfomycin, trimethoprim and co-trimoxazole. Nitrofuran derivatives displayed higher antimicrobial activity than other antimicrobial agents regardless of bacteria species or resistance mechanism.

Keywords: ESBL-positive *E. coli*; MRSA; furazidin; nitrofurantoin; urinary tract infections

Urinary tract infections are a severe public health problem and remain one of the most common community-acquired bacterial infections. Community-acquired urinary tract infections affect approximately 10-20% of the population, while hospital-acquired urinary tract infections account for approximately 30-40% of all nosocomial infections (1-3).

Urinary tract infections are caused by a wide range of pathogens, including gram-negative and gram-positive bacteria and fungi. The predominant causative agent of both uncomplicated and complicated urinary tract infections is *E. coli*, especially uropathogenic strains. Others aetiological agents of uncomplicated urinary tract infections include gram-negative rod-shaped bacteria of the Enterobacteriaceae, such as *Proteus mirabilis*, *Klebsiella pneumoniae*, *Citrobacter* spp., and *Enterobacter* spp., as well as gram-positive cocci, such as *Staphylococcus saprophyticus* and *Enterococcus* spp. Complicated urinary tract infections occur in patients with functional or anatomical obstructions of urine-flow or host defense dysfunctions and are caused by the gram-negative bacteria *K. pneumoniae*, *P. mirabilis*, *Enterobacter* spp., *Serratia marcescens*, *Pseudomo-*

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nas aeruginosa, and *Acinetobacter* spp., as well as the gram-positive cocci: *Enterococcus* spp. and *S. aureus* (2-5).

The Infectious Diseases Society of America (IDSA) and the European Society for Clinical Microbiology and Infectious Diseases (ESCMID) as well as polish Recommendations for diagnosis, therapy and prevention of urinary tract infections in adults edited by Hryniewicz and Holecki in 2015, recommend nitrofurantoin, fosfomycin, co-trimoxazole, beta-lactams (especially amoxicillin/clavulanic acid) or fluoroquinolones in treatment of uncomplicated urinary tract infections (3, 6).

The main reason for treatment failure in these infections is the increasing resistance of uropathogens to commonly used antibiotics and chemotherapeutics. Among strains isolated from nosocomial and community-acquired infections, extended-spectrum beta-lactamases (ESBL) producing Enterobacteriaceae are the most frequently isolated. Extended-spectrum beta-lactamases with substantial potential to hydrolyse beta-lactam rings are able to hydrolyse penicillins, cephalosporins (but not cephamycins, e.g. cefoxitin), and monobactam antibiotics. Moreover, ESBL production is often accompanied by resistance to fluoroquinolones, co-trimoxazole or aminoglycosides (3, 4, 7-9). The reason for treatment failures of urinary tract infections caused by gram-positive bacteria results from resistance to glycopeptides of *Enterococcus* spp. and to methicillin of staphylococci strains. Resistance to methicillin reduces the activity of all beta-lactam antibiotics activity and is often associated with resistance to macrolides, lincosamides and streptogramins B (MLS_B antibiotics) (10) and a decreased susceptibility to aminoglycosides and quinolones (3, 4, 11). It is worth noting that emergence of MDR among uropathogens, defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, additionally limits the antibiotics options (12).

In the era of progressive increases of bacterial resistance to antimicrobial agents, a re-evaluation of the efficacy of older generations of antimicrobial agents, such as nitrofuran derivatives (e.g. nitrofurantoin and furazidin, Fig. 1), may provide additional information on their treatment potential.

The therapeutic efficacy of nitrofuran derivatives is the consequence of their multidirectional mechanisms of action, which impact various processes crucial for bacterial cell functions. The first pathway involves metabolic reduction by nitroreductases to form highly reactive intermediates such as nitrosamine and/or hydroxylamine derivatives, and aromatic amine derivatives. These metabolic intermediates fully inhibit the synthesis of proteins in bacterial cells. The second path consist of the one-electron reduction of nitro groups to generate nitroanions, which are responsible for the oxidation of oxygen to superoxide radical anions. The resulting radicals damage bacterial cells, causing permanent structural changes to DNA, RNA, and mitochondria. It thus seems that the extended mechanism of action of nitrofuran derivatives might be responsible for lower drug resistance in comparison to other antibiotics (13-15).

Nitrofurantoin has recently been the subject of intensive evaluation towards different pathogens responsible for urinary tract infections (13, 14). However, current knowledge of the antimicrobial activity of furazidin is lacking and there is an insufficient data on the susceptibility of different bacterial species to furazidin. Thus, the aim of this study was to evaluate furazidin activity against common uropathogens (Enterobacteriaceae strains, gram-positive cocci and anaerobic bacteria) in comparison to nitrofurantoin and other selected antimicrobial agents (ciprofloxacin, fosfomycin, trimethoprim and co-trimoxazole) routinely used in the treatment of urinary tract infections. We also evaluated the activity of furazidin against MDR strains of *E. coli* and *S. aureus* and performed a preliminary comparative kinetic study of furazidin and nitrofurantoin activities.

EXPERIMENTAL

Bacterial strains

The analysis of the activities of selected antimicrobial agents was performed against a total

Figure 1. Chemical structure of nitrofurantoin (E)-1-[(5-nitro-2-furyl)methylideneamino]imidazolidine-2,4-dione and furazidin (1-{[3-(5-nitro-2-furyl)prop-2-en-1-ylidene]amino}imidazolidine-2,4-dione)

of 46 strains, including 34 clinical strains and 12 reference strains: 10 strains of ATCC collection and two of other collections (BAA, NCIMB). Among gram-negative bacteria of Enterobacteriaceae 24 strains were tested: 18 strains clinical ESBL-positive *E. coli* and six reference strains, such as: *E. coli* ATCC 25922, *E. coli* ATCC 35218, *K. pneumoniae* ATCC 700603, *Enterobacter aerogenes* NCIMB 10102, *P. mirabilis* ATCC 12453 and *Salmonella enterica subsp. enterica serovar* Agona ATCC 51957. The analysis was also conducted against 20 gram-positive cocci including 16 clinical MRSA isolates and additionally four reference strains: *S. aureus* ATCC 25923, *S. aureus* BAA 976, *S. epidermidis* ATCC 12228, *Enterococcus faecalis* ATCC 29212. Moreover, anaerobic reference strains: *Bacteroides fragilis* ATCC 25285, *Prevotella loescheii* ATCC 15930 were included to the study.

Among *E. coli* strains, five were isolated from outpatients who suffered urological complications after invasive procedures (e.g. catheterization) and 13 isolates from patients hospitalized in the intensive care unit. Species identification, susceptibility testing and determination of ESBL activity via the double-disc method (16) and via PCR were all performed. In total, seven *S. aureus* strains were isolated from subjects of the outpatient clinic, while nine isolates from patients hospitalized in the intensive care unit. The identification of species and susceptibility testing of MRSA and MLS_B resistant *S. aureus* strain were also performed.

PCR based diagnosis of ESBL

Bacterial genomic DNA was isolated with the use of commercial kit (Genomic Mini, A&A Biotechnology, Poland) according to the manufacturer's instructions. The amplification studies included three separate reactions, one for each beta-lactamase encoding gene: $bla_{CTX\text{-}M\text{-}1}$, bla_{SHV} and bla_{TEM}.

The $bla_{CTX\text{-}M\text{-}1}$, bla_{SHV} and bla_{TEM} related genes were respectively amplified by using the following primer pairs: P1C (TTAATTCGTCTCTTCCAGA) and P2D (CAGCGCTTTTGCCGTCTAAG) (17), SHV-A (ACTGAATGAGGCGCTTCC) and SHV-C (CGCACCCCGCTTGCT), and TEM-A (ATAAAA TTCTTGAAGAC) and TEM-B (TTACCAAT-GCTTAATCA) (9). PCR was performed in thermo-

Table 1. Antimicrobial susceptibility testing of clinical Escherichia coli strains against antimicrobials recommended in urinary tract infections treatment.

No.	Strain	Origin	Resistance mechanisms		Antimicrobial susceptibility testing			
			Fenotyping	Genotyping	Ciprofloxacin	Fosfomycin	Trimethoprim	Co-trimoxazole
1	*E. coli* 1 24950	CA [1]	ESBL	CTX-M-1	R [3]	R	R	R
2	*E. coli* 2 25251	CA	ESBL	CTX-M-1	R	R	R	R
3	*E. coli* 3 25080	CA	ESBL	*undefined*	R	S	R	R
4	*E. coli* 4 27206	CA	ESBL	CTX-M-1	R	S	R	R
5	*E. coli* 5 33685	CA	ESBL	CTX-M-1	S [4]	S	S	S
6	*E. coli* 12	HA [2]	ESBL	CTX-M-1	R	R	S	S
7	*E. coli* 304	HA	ESBL	CTX-M-1	R	S	R	R
8	*E. coli* 467	HA	ESBL	CTX-M-1	R	S	R	R
9	*E. coli* 540	HA	ESBL	CTX-M-1	R	S	R	R
10	*E. coli* 640	HA	ESBL	CTX-M-1	R	S	R	R
11	*E. coli* 672	HA	ESBL	*undefined*	R	S	S	S
12	*E. coli* 1267	HA	ESBL	TEM, CTX-M-1	R	S	R	R
13	*E. coli* 1361	HA	ESBL	CTX-M-1	R	S	R	R
14	*E. coli* 1665	HA	ESBL	CTX-M-1	R	S	R	R
15	*E. coli*	HA	ESBL	CTX-M-1	R	S	R	R
16	*E. coli* 2032	HA	ESBL	CTX-M-1	R	S	R	R
17	*E. coli* 3086	HA	ESBL	TEM	R	S	S	S
18	*E. coli* 4191	HA	ESBL	SHV	R	S	S	S
No. (percentage) of resistant strains					17 (94.4%)	3 (16.6%)	13 (72.2%)	13 (72.2%)

[1]CA - community acquired; [2]HA - hospital acquired; [3]R - resistant; [4]S - susceptible

cycler T personal (Biometra, Germany). The conditions for the $bla_{CTX-M-1}$ and bla_{SHV} amplifications were as follow: initial denaturation at 94°C for 2 min, 30 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 45 s and a final elongation at 72°C for 5 min. The bla_{TEM} PCR amplification conditions were as follows: 94°C for 3 min, 30 cycles of 94 C for 15 s, 42°C for 30 s, 72°C for 30 s and a final elongation at 72°C for 7 min. All amplicons were subjected to electrophoresis in a 2% agarose gel and stained with ethidium bromide.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of clinical isolates against ciprofloxacin, fosfomycin, trimethoprim and co-trimoxazole were performed and analysed according to the EUCAST recommendations (18).

The efficacy of nitrofuran derivatives was assessed by determining minimal inhibitory concentrations using the broth microdilution or agar dilution method (19). The interpretation of susceptibility was carried out for nitrofurantoin according to the EUCAST clinical breakpoints (18). The MIC breakpoints for furazidin were not established. Furazidin is often incorrectly administered to the patients, based on nitrofurantoin susceptibility (3).

The broth microdilution method was conducted to obtain MIC values of aerobic bacterial strains. An inoculum of 1 McFarland in sterile 0.85% NaCl solution was prepared from a pure bacterial culture grown for 24 h. The analysis was conducted in a 96-well microtiter plate. Bacterial inoculums were aliquoted into the wells with 150 µL of Mueller Hinton Broth (MHB) (Oxoid, UK) containing the tested antimicrobial compounds at appropriate dilutions. The concentration of the bacterial cells in each well was approximately 10^6 CFU/mL. The positive control was MHB inoculated with bacterial suspension. The plates were incubated in an orbital shaker incubator (150 rpm, 37°C, aerobic atmosphere, 24 h). Two replicate samples were tested in double repeats for each condition to ensure repeatability. The results were analysed in relation to the absorbance of the positive control. The MIC values were considered to be the lowest antimicrobial concentration having an absorbance level = 0.1. The optical densities were

Table 2. Activity of furazidin and nitrofurantoin against clinical Enterobacteriaceae strains obtained by the broth microdilution method.

No.	Strain	MIC [mg/L]	
		Furazidin	Nitrofurantoin
1	*E. coli* 124950	16	32
2	*E. coli* 2 25251	8	32
3	*E. coli* 3 25080	8	16
4	*E. coli* 4 27206	4	16
5	*E. coli* 5 33685	8	16
6	*E. coli* 12	8	16
7	*E. coli* 304	8	16
8	*E. coli* 467	8	16
9	*E. coli* 540	8	32
10	*E. coli* 640	8	16
11	*E. coli* 672	16	32
12	*E. coli* 1267	64	32
13	*E. coli* 1361	8	32
14	*E. coli* 1665	16	32
15	*E. coli* 1913	8	16
16	*E. coli* 2032	8	16
17	*E. coli* 3086	8	16
18	*E. coli* 4191	16	32
	MIC_{50}	8	16
	MIC_{90}	16	32

Table 3. Activity of furazidin and nitrofurantoin against reference Enterobacteriaceae strains obtained by the broth microdilution method.

No.	Strain	MIC [mg/L]	
		Furazidin	Nitrofurantoin
1	*E. coli* ATCC 25922	8	16
2	*E. coli* ATCC 35218	8	16
3	*K. pneumoniae* ATCC 700603	32	64
4	*P. mirabilis* ATCC 12453	32	64
5	*E. aerogenes* NCIMB 10102	32	32
6	*S. enterica* ATCC 51957	32	32

Table 4. Antimicrobial susceptibility testing of clinical *Staphylococcus aureus* strains against antimicrobials recommended in urinary tract infections treatment.

No.	Strain	Origin	Resistance mechanisms	Antimicrobial susceptibility testing			
				Ciprofloxacin	Fosfomycin	Trimethoprim	Co-trimoxazole
1	*S. aureus* 8	CA [1]	MRSA, MLS_B	S [4]	S	S	S
2	*S. aureus* 808	CA	MRSA, MLS_B	R [3]	S	S	S
3	*S. aureus* 991	CA	MRSA, MLS_B	R	S	S	S
4	*S. aureus* 2035	CA	MRSA, MLS_B	S	S	S	S
5	*S. aureus* 2418	CA	MRSA, MLS_B	R	S	R	R
6	*S. aureus* 4836	CA	MRSA, MLS_B	R	S	R	R
7	*S. aureus* 6718	CA	MRSA, MLS_B	S	S	S	S
8	*S. aureus* 212	HA[2]	MRSA	S	S	S	S
9	*S. aureus* 375	HA	MRSA	S	S	S	S
10	*S. aureus* 385	HA	MRSA, MLS_B	R	S	S	S
11	*S. aureus* 403	HA	MRSA, MLS_B	R	S	S	S
12	*S. aureus* 466	HA	MRSA, MLS_B	S	S	S	S
13	*S. aureus* 511	HA	MRSA, MLS_B	R	S	R	S
14	*S. aureus* 675	HA	MRSA	S	S	S	S
15	*S. aureus* 728	HA	MRSA, MLS_B	R	S	R	
16	*S. aureus* 2110	HA	MRSA, MLS_B	R	S	S	S
	No. (percentage) of resistant strains			9 (56.3%)	0 (0%)	4 (25%)	3 (18.8%)

[1]CA – community acquired; [2]HA – hospital acquired; [3]R – resistant; [4]S – susceptible

measured at 600 nm (OD_{600}) using a spectrophotometer (Tecan SunRise, Switzerland).

Agar dilution method were performed for anaerobic bacterial strains. Cells from pure cultures (grown for seven days) were resuspended in sterile 0.85% NaCl solution (bioMérieux, France) to obtain an equivalent of 1 McFarland. The inoculum was spread onto Schaedler Agar (Oxoid, UK) with 5% sheep blood plates containing appropriate dilutions of the antimicrobial agents and was incubated in an anaerobic atmosphere (GenBag anaer, bioMérieux, France) at 37°C for seven days. The MICs were defined as the lowest dilution that inhibited bacterial growth. All tests were conducted in duplicate.

Kinetic assay of furazidin and nitrofurantoin activities against *S. aureus* and *E. coli* strains

To assess the effect of furazidin and nitrofurantoin on bacterial growth, 18 h assay were performed. In total, 7 concentrations of nitrofuran derivatives were prepared (0.5 × MIC – 16 × MIC) in MHB with final volumes of 200 µL. Each well

was inoculated with bacterial suspension to obtain a concentration of approximately 10^6 CFU/mL. The optical density was measured at 600 nm in 2 h intervals for 18 h.

RESULTS

The selected nitrofuran derivatives furazidin and nitrofurantoin were evaluated *in vitro* for their antimicrobial activity against resistant and susceptible strains of Enterobacteriaceae, gram-positive cocci and anaerobic bacteria. In addition, the susceptibility to ciprofloxacin, fosfomycin, trimethoprim and co-trimoxazole of clinical *E. coli* and *S. aureus* strains were assessed.

Activities of furazidin and nitrofurantoin against Enterobacteriaceae strains

Furazidin and nitrofurantoin were tested for their antimicrobial activities against 24 Enterobacteriaceae strains.

Clinical strains

All 18 clinical *E. coli* strains were identified as ESBL-positive according to the phenotypic double-disc synergy test as well as beta-lactamase production were confirmed by PCR. The molecular studies concerning the presence of selected ESBL encoding genes revealed that 14 *E. coli* strains carried the genes belonging to the $bla_{\text{CTX-M-1}}$ group, one to the bla_{TEM} and one to the bla_{SHV}. Within the analysed strains the co-existence of $bla_{\text{CTX-M-1}}$ and bla_{TEM} was observed in one isolate while two strains possessed undefined ESBL genotypes. Among analysed strains, the resistance rate amount to 94.4% for ciprofloxacin, 72.2% for trimethoprim, 72.2% for co-trimoxazole and 16.6% for fosfomycin (Table 1). In contrary, all 18 tested *E. coli* strains remained within the susceptibility criteria for nitrofurantoin, according to the EUCAST clinical breakpoints.

Nitrofuran derivatives displayed higher activities against MDR *E. coli* strains than other tested antimicrobial agents. The MIC values for furazidin obtained by the broth microdilution method for clinical *E. coli* strains ranged from 4 mg/L to 64 mg/L (MIC_{50} amounted to 8 mg/L and MIC_{90} – 16 mg/L) while the MICs for nitrofurantoin ranged from 16 mg/L to 32 mg/L (MIC_{50} amounted to 16 mg/L and MIC_{90} – 32 mg/L). The comparison of MIC_{50} and MIC_{90} values showed better activity of furazidin

Table 5. Activity of furazidin and nitrofurantoin against tested clinical gram-positive strains obtained by the broth microdilution method.

No.	Strain	MIC [mg/L]	
		Furazidin	Nitrofurantoin
1	*S. aureus* 8	4	16
2	*S. aureus* 808	4	16
3	*S. aureus* 991	4	16
4	*S. aureus* 2035	4	16
5	*S. aureus* 2418	4	16
6	*S. aureus* 4836	2	8
7	*S. aureus* 6718	4	16
8	*S. aureus* 212	4	16
9	*S. aureus* 375	4	16
10	*S. aureus* 385	4	32
11	*S. aureus* 403	4	32
12	*S. aureus* 466	4	16
13	*S. aureus* 511	4	16
14	*S. aureus* 675	4	16
15	*S. aureus* 728	4	16
16	*S. aureus* 2110	4	16
	MIC_{50}	4	16
	MIC_{90}	4	16

Table 6. Activity of furazidin and nitrofurantoin against selected reference gram-positive strains obtained by the broth microdilution method.

No.	Strain	MIC [mg/L]	
		Furazidin	Nitrofurantoin
1	*S. aureus* BAA 976	4	16
2	*S. aureus* ATCC 25923	4	64
3	*S. epidermidis* ATCC 12228	2	32
4	*E. faecalis* ATCC 29212	4	32

Table 7. MIC values of furazidin and nitrofurantoin against reference strains of anaerobic bacteria obtained by the agar dilution method.

Anaerobic strains	MIC values [mg/L]	
	Furazidin	Nitrofurantoin
B. fragilis ATCC 25285	0.5	4
P. loescheii ATCC 15930	0.5	4

than nitrofurantoin against MDR *E. coli* isolates (Table 2).

Reference strains

Moreover, the nitrofuran derivatives were evaluated against six Enterobacteriaceae strains belonging to ATCC and NCIMB collections. The MIC values for furazidin obtained by the broth microdilution method for reference gram-negative strains ranged from 8 mg/L to 32 mg/L while the MICs for nitrofurantoin ranged from 16 mg/L to 64 mg/L. Furazidin and nitrofurantoin exhibited similar activities against *E. aerogenes* NCIMB 10102 strain. It is worth noting, that furazidin showed 2-fold higher activity than nitrofurantoin against *P. mirabilis*, which remains naturally resistant to nitrofurantoin (20). Similarly, 2-fold lower MIC for furazidin than nitrofurantoin for *E. coli* and *K. pneumoniae* was observed (Table 3).

Activities of furazidin and nitrofurantoin against strains of gram-positive bacteria

Clinical strains

Among 16 tested MRSA strains, 13 presented co-existence with the MLS_B resistance mechanisms (Table 4). Moreover, 56.3% of strains were resistant to ciprofloxacin, 25% to trimethoprim and 18.8% to co-trimoxazole (Table 4).

Among evaluated nitrofuran derivatives furazidin displayed higher activity against gram-positive cocci than nitrofurantoin. MIC values obtained for furazidin using the broth microdilution method ranged from 2 to 4 mg/L (MIC_{50} and MIC_{90} amounted to 4 mg/L), while MIC values for nitrofurantoin ranged from 8 to 32 mg/L (MIC_{50} and MIC_{90} amounted to 16 mg/L) (Table 5). The comparison of MIC_{50} and MIC_{90} values showed better activity of furazidin than nitrofurantoin against MDR *S. aureus* isolates.

Reference strains

MIC values obtained for furazidin for reference gram-positive strains ranged from 2 to 4 mg/L, while MIC values for nitrofurantoin ranged from 16 to 64 mg/L. Furazidin showed higher antibacterial activity than nitrofurantoin against *S. aureus* BAA 976, *S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228 and *E. faecalis* ATCC 29212 (Table 6).

Activities of furazidin and nitrofurantoin against reference strains of anaerobic bacteria

Both nitrofuran derivatives furazidin and nitrofurantoin had low MIC values against anaerobic bacteria strains. However, furazidin displayed lower MIC values than nitrofurantoin (0.5 mg/L *vs* 4 mg/L) (Table 7).

Preliminary kinetic assay of furazidin and nitrofurantoin activities against *S. aureus* and *E. coli* strains

Analyses of antibiotic or chemotherapeutic MICs were based on the OD_{600} values with the cut-off point set at an OD_{600} value of 0.1. The graphs show the changing density of the bacterial culture during exposure to different drug concentrations. Representative curves for *E. coli* 4 and MRSA 511 show bacterial

growth in the presence of increasing concentrations of furazidin and nitrofurantoin. The maximal bacterial density (maximum OD_{600} value) of drug-free control occurred after 8 h of incubation (Fig. 2).

DISCUSSION AND CONCLUSION

Antimicrobial resistance among bacteria isolated from community-acquired or nosocomial urinary tract infection has been steadily increasing, especially for beta-lactams, co-trimoxazole and fluoroquinolones (6, 14). At the same time a diminishing number of approvals for new antimicrobials active against uropathogens has been observed (14). Thus, in the era of increasing bacterial resistance, the re-evaluation of nitrofurans is a potential way to obtain drugs for the successful therapy of urinary tract infections caused by strains resistant to other commonly used drugs, especially by MDR bacterial strains.

In the USA, an alarming problem exists where a high prevalence of outpatient MDR *E. coli* strains, which has markedly grown from 9.1% in 2001 to 17% in 2010 (21). According to the surveillance study carried out in Europe and Brazil between 2006 and 2008 of the antimicrobial resistance of pathogens from uncomplicated urinary tract infections, 48.2% of *E. coli* strains were resistant to ampicillin, 29.4% to co-trimoxazole and 8.1% to ciprofloxacin (22). In Poland, between 2006 and 2008, the resistance rate of *E. coli* isolated from uncomplicated cases of urinary tract infections was 40% for ampicillin, 20% for co-trimoxazole and 6.7% for ciprofloxacin. Among isolates from nosocomial urinary tract infections, resistance to antimicrobial agents was higher, with 56.8% of strains being resistant to ampicillin, 35% to tetracycline, 23.1% to co-trimoxazole to 19.4% to ciprofloxacin (3, 22, 23). The prevalence of resistance to nitrofurantoin among *E. coli* strains is relatively low compared to other antimicrobial agents, with only 2% of isolates in Europe and USA showing resistance. In Poland, 4.4% of *E. coli* strains isolated from community-acquired urinary tract infections were resistant to nitrofurantoin, as well as 3.75% of isolates from nosocomial infections (3, 22-24).

Despite the increasing bacterial resistance to antimicrobial agents, nitrofurantoin remains a good option in the treatment of community-acquired urinary tract infections caused by *E. coli*. Nitrofurantoin has maintained good antimicrobial activity despite its extensive clinical use worldwide for over 50 years, and it still possesses good bactericidal activity against MDR *E. coli* (7, 13-15, 21, 24-26) as well as vancomycin-resistant enterococci strains (11, 13, 15).

The main purpose of this study was to re-investigate the antimicrobial activities of selected nitrofuran derivatives against common uropathogens. Our

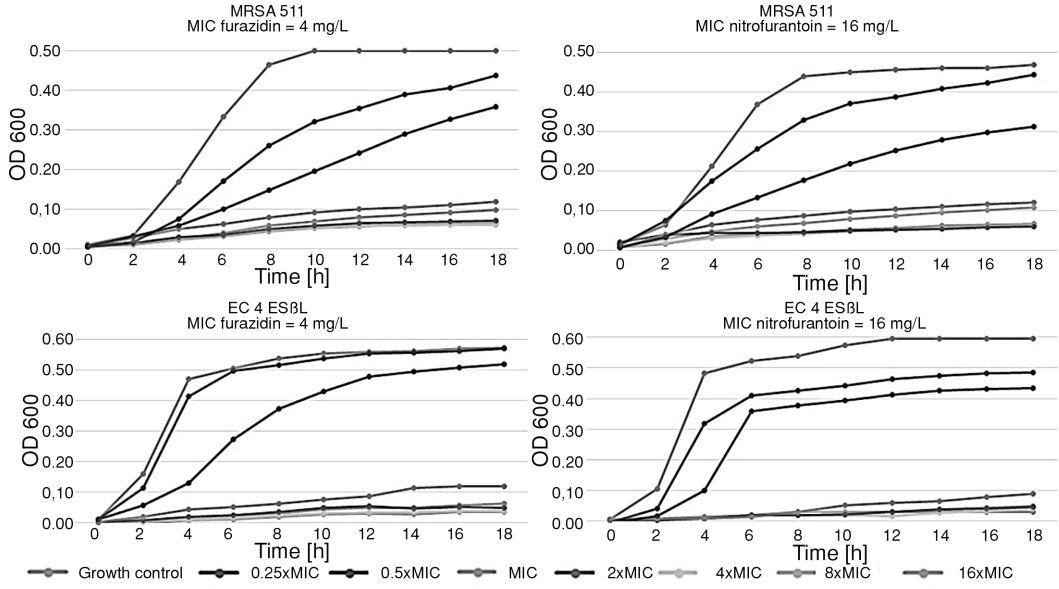

Figure 2. Representative growth curves of clinical MDR strains: *Escherichia coli* 4 and MRSA 511 at different concentrations of furazidin and nitrofurantoin

study involved the evaluation of furazidin and nitrofurantoin against 46 strains: 34 clinical MDR strains of *E. coli* and *S. aureus* and 12 reference strains: 10 strains of ATCC collection and two of other collections (BAA, NCIMB). Generally, evaluated nitrofuran derivatives displayed higher antimicrobial activity in comparison to the other tested antimicrobial agents (ciprofloxacin, trimethoprim and co-trimoxazole) regardless of the species of bacteria or their resistance mechanism. It was shown that nitrofuran derivatives retained their activity against all clinical MDR strains of *E. coli* and *S. aureus*. Among other tested antimicrobials, fosfomycin demonstrated good antimicrobial activity and was active against all *S. aureus* strains and against 83.4% of *E. coli* strains (Table 1, Table 4).

While much research has been focused on activity of nitrofurantoin against MDR urinary *E. coli* isolates, furazidin might be regarded as a valuable alternative. Comparative analyses of antibacterial properties revealed higher activity of furazidin in comparison to nitrofurantoin, expressed as lower MIC values. Our results are still consistent with previous outcomes and confirmed lower MIC values for furazidin than nitrofurantoin (27). The comparison of MIC values for furazidin and nitrofurantoin for all strains showed that only one strain, ESBL-positive *E. coli* 1267, expressed a higher MIC value for furazidin than nitrofurantoin (64 *vs* 32 mg/L). It is worth noting that this strain remained susceptible for nitrofurans according to the EUCAST interpretation criteria. Moreover, furazidin displayed higher antimicrobial activity against *P. mirabilis* and *K. pneumoniae* in comparison to nitrofurantoin.

Nitrofuran derivatives expressed good antibacterial activity against MDR *S. aureus* strains. While *S. aureus* is unfrequently isolated from community-acquired urinary tract infections, among hospitalized patients is more common (3, 28). MDR *S. aureus* is predominantly recovered from long-term hospitalized patients with urinary tract catheterization. Moreover, it is hypothesized that *S. aureus* urinary tract infection may lead to invasive infection or staphylococcal bacteraemia (28). Our research revealed better activity of furazidin than nitrofurantoin against MDR *S. aureus* expressed by 4-fold lower MIC_{50} and MIC_{90} of furazidin than nitrofurantoin (4 mg/L *vs* 16 mg/L, respectively).

Moreover, Zhanel et al. reported nitrofurantoin to be a good therapeutic option in the treatment of vancomycin-resistant enterococci isolated from urinary tract infection (11). Our study revealed high activity of nitrofuran derivatives especially, furazidin against enterococci (Table 6).

In summary, the increase in bacterial resistance to antibiotics and chemotherapeutics used in the treatment of urinary tract infections is encouraging initiatives to re-investigate the antimicrobial activity of older generation of drugs. Herein evaluated furazidin and nitrofurantoin showed higher antimicrobial activity than ciprofloxacin, trimethoprim and co-trimoxazole regardless of the species of bacteria or the resistance mechanism being tested. Our results suggest that ciprofloxacin, trimethoprim and co-trimoxazole should be carefully used in the therapy of urinary tract infections, regard to the results of antimicrobial susceptibility testing. Moreover, nitrofuran derivatives displaying higher antimicrobial activity in comparison to other antimicrobials, might be considered as a good first line therapy of urinary tract infections against a broad-spectrum of uropathogens, including MDR strains of *E. coli* and *S. aureus*.

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